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PROVISIONAL SPECIFICATION

Improvements in or relating to the Fractionation of the Components of a Mixture in Solution

We, STANLEY MILES PARTRIDGE, ROLAND GORDON WESTALL and JAMES REGINALD BENDALL, all of the Low Temperature Research Station, Downing Street, Cambridge, all British Subjects, do hereby declare the nature of this invention to be as follows:—

Methods of fractionation of the components of a mixture in solution by differential adsorption in a filter column of an adsorbent such as alumina, active clay or active charcoal are well known, including so-called flowing methods and a particular development (due to Tiseleus) applicable to substances which are not irreversibly adsorbed consists in first forcing a small volume of the solution into the top of the column and then washing with a solution of substance (the " developer ") which is more strongly adsorbed than any of the components of the mixture. The latter is thus forced to move in front of the front of the developer, and is separated into different zones, each of which contains one component. Thus as the flow of developer solution is continued, the fractions constituting the zones flow out of the column in turn and if the components are known, it suffices to measure some physical property of the outflowing solution which varies with the components, e.g. the refractive index. By plotting the results (which by suitable apparatus can be done continuously) a curve with quite sharp steps is obtained, the dimension of the steps along one axis constituting a qualitative analysis and along the other axis constituting a quantitative analysis.

The present invention is concerned with a similar method of fractionation, but instead of using a filter column depending on a purely physical effect, it uses a column having a reversible chemical effect, the developer being a substance which has a stronger affinity for the column material than has any of the components to be separated. Ion-exchange materials constitute one class of material which can be used in this way, in particular synthetic resinous ion-exchange materials, and the invention will be further described by reference to such materials, which are used as a column packing in the form of particles graded by sieving.

The solution containing the mixture of organic substances which may also contain in organic salts, is passed at a uniform speed through the column, and those ions, acids or bases, that are reactive towards the ion-exchanger used for packing the column, are absorbed or combined, while ions of opposite charge or electrically neutral substances flow out with the solvent. The solution is allowed to pass until a certain proportion of the ion-exchange material is saturated and will absorb no more, when the flow of solution is discontinued. At this point a boundary has formed some distance along the tube, which divides the saturated from the unsaturated portion of the column, and to some extent the absorbed components of the solution have arranged themselves in order of decreasing affinity for the ion-exchanger, the most strongly combined substances appearing in the first part of the column. A developer in the form of a solution containing an appropriate concentration of a substance which is more strongly combined than any of the substances to be separated, and which may be either an organic or an inorganic acid or base, is then passed through the column at a regulated rate. This substance forms a sharply bounded band at the beginning of the column displacing the most strongly combined component of the original solution. This in turn displaces the next component, until a time is reached at which the system acquires a dynamic equilibrium and the components form a series of sharply separated bands, all moving along the column together. When the least strongly combined component reaches the end of

the column it flows out with the solvent.

Since in general the components will not be coloured substances and the separate bands formed by the individual solutes on the column will not be visible, a means is necessary whereby the composition of the effluent liquid may be continuously observed, in order to determine at what time it is necessary to change the receiver collecting the effluent so as to separate clean fractions. For this purpose measurement of one or more of the physical properties of the components may be used. The most useful of these properties are electrical conductivity, hydrogen ion concentration and refractive index, but in particular cases other properties may be utilised; the most suitable physical properties to be measured must be chosen in relation to the properties of the components in the solution, each case being considered on its merits.

For the properties referred to, the apparatus is completed by the provision of suitable instruments for the measurement of electrical conductivity, hydrogen ion concentration and refractive index of the liquid flowing from the column, these instruments to be arranged either to give a continuous indication or to make a permanent record on a graph or photographic film of the changes in the properties measured. An arrangement is also required whereby the volume of each fraction may be measured or its weight observed, and switch gear may be incorporated to change the receiver automatically as a new fraction appears.

The ion exchange material may be a substance which is capable of forming insoluble salt-like complexes with acids, or may be similarly reactive with bases and the insoluble salts so formed should possess the property of reversible dissociation, so that weakly reacting ions, acids or bases, may be quantitatively displaced under the proper conditions by those which have a stronger affinity for the ion exchange material. Synthetic resins prepared by the polymerization of smaller molecules having an acidic or basic function are suitable.

The solution containing the mixture of organic substances to be separated may be an aqueous extract or may contain varying proportions of organic solvents miscible with water such as alcohol or acetone; the use of organic solvents in this way may be necessary where some of the components to be separated have low solubilities in water alone. When the solution contains mixed organic and inorganic salts, as is very common with extracts of biological origin, the solution may be first passed through a column containing a synthetic resin with basic properties which removes anions and leaves a solution containing free bases, both organic and inorganic, together with non-electrolytes. The effluent solution may then be passed through a column with acid properties which removes the bases, the non-electrolytes flowing through with the solvent. Solutions more suitable for separation may then be obtained from the columns, by passage of an acidic developer in the one case and a basic developer in the other: by suitable fractionation a solution containing organic acids only may be recovered from the column with basic properties and a similar fraction containing organic bases only may be obtained from the column with acid properties, the two solutions then being dealt with separately on the appropriate columns.

It should be mentioned that efficiency of separation is directly related to the height of the band occupied by the individual to be separated. For good separation on laboratory columns this height should be as much as 10 cms. so that for solutions containing a large number of components the columns become inconveniently long. We find however that the desired effect can be obtained by attaching a narrow subsidiary column packed with fine material to the bottom of the main column. The main column can then be shorter and wider and is consequently more easily packed and handled.

The following example will further illustrate the nature of the invention:—

A column was packed with 3 gms. of a synthetic cation-exchange resin and mounted above an assembly consisting of a conductivity cell and a glass electrode cell, both arranged for the continuous measurement of the conductivity and *p*H of the effluent from the column, the two cells and a socket for the column all being built of methyl-methacrylate plastic. The ion-exchange material was activated by the passage of 2 N hydrochloric acid, and was then washed with distilled water until the conductivity and *p*H readings showed that the effluent was substantially free from hydrochloric acid.

A mixture containing creatinine (192 mgs.) and glycine (113 mgs.) dissolved in 20 ml. of water was then passed into the column. The substances were adsorbed in the upper part of the column and as both test substances had the property of modifying the brown colour of the ion-exchange material, their presence was shown by the formation of two bands, one of them orange-yellow in colour and the other red-brown. In order to remove

traces of anions present as impurities in the test substances, the columns were then washed with distilled water (50 mls.) until the conductivity of the effluent fell to a value approaching that of the distilled water used for washing.

Ammonia solution (0.0213 N) was then passed into the column in order to displace the bands due to the test substances and to cause their passage down the column. The part of the column occupied by the ammonia was dark brown in colour, and as they passed down the column, the fronts due to the three substances were observed to become more sharp.

From the time the ammonia solution was applied to the first appearance of glycine in the effluent 275 mls. of substantially pure water flowed from the column. From then on to the final appearance of pure ammonia solution in the effluent, recordings of resistance in ohms across the conductivity cell and millivolts across the glass electrode cell were taken at intervals of 1 ml. and the results were plotted. As the glycine increased in concentration when its front reached the lower orifice of the column the resistance rose to 4.75×10^4 ohms. At this value it remained substantially stationary until the front due to creatinine reached the orifice. A marked fall in resistance followed and resistance measurements again became stationary when the last of the glycine left the column and the effluent consisted of pure creatinine solution only. Similarly, the resistance again fell sharply and then reached a further stable level as the ammonia boundary reached the bottom of the column.

Since the test solutions were displaced in order of increasing strength as bases, the *p*H measurements increased in steps, the shape of the curve being the inverse of that given by the measurements of resistance.

In order to verify the purity of the fractions lying under the flat portions of the curves, analyses were carried out on appropriate fractions of the effluent. The recovery of creatinine in a pure condition was 63.5 percent. and the total recovery was 99 percent.; while the recovery of pure glycine was 74.5 percent., the total recovery being 100 percent.

It should be pointed out that the percentage recovery of the pure components shown in this experiment should not be taken as an indication of the probable efficiency of industrial applications of the method since the apparatus used was designed for the separation of small quantities of material as a laboratory micro-method. Efficiency is considerably improved by use of longer columns and larger quantities of material.

Dated this 29th day of May, 1947.
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15, Great James Street, London, W.C.1,
Agents for the Applicants.

COMPLETE SPECIFICATION

Improvements in or relating to the Fractionation of the Components of a Mixture in Solution

We, STANLEY MILES PARTRIDGE, ROLAND GORDON WESTALL and JAMES REGINALD BENDALL, all of the Low Temperature Research Station, Downing Street, Cambridge, all British Subjects, do hereby declare the nature of this invention and in what manner the same is to be performed, to be particularly described and ascertained in and by the following statement:—

Methods of fractionation of the components of a mixture in solution by differential adsorption in a filter column of an adsorbent such as alumina, active clay or active charcoal are well known, including so-called flowing methods and a particular development (due to Tiseleus) applicable to substances which are not irreversibly adsorbed consists in first passing a small volume of the solution into the top of the column and then washing with a solution of substance (the "developer") which is more strongly adsorbed than any of the components of the mixture. The latter is thus forced to move in front of the front of the developer, and is separated into different zones, each of which contains one component. Thus as the flow of developer solution is continued, the fractions constituting the zones flow out of the column in turn and if the components are known, it suffices to measure some physical property of the outflowing solution which varies with the components, e.g. the refractive index. By plotting the results (which by suitable apparatus can be done continuously) a curve with quite sharp steps is obtained, the dimension of the steps along one axis constituting a qualitative analysis and along the other axis constituting a quantitative analysis.

Processes have been described for the separation of organic solutes of biological origin from solutions containing mixtures thereof by passing the solution through a column of ion-exchange material, in particular, an ion-exchange synthetic resin, the solutes being adsorbed by the ion-exchange material and then desorbed and separated from one another by successive treatment with different desorbing agents or desorbing agents of different strengths. Such process make use of a reversible chemical effect, exhibited by ion-exchange materials and in particular synthetic resinous ion-exchange materials.

The present invention is concerned with a method of fractionation of solutions containing mixed solutes consisting at least in part of solutes of biological origin, similar to Tiseleus flowing method above described, but, as in the other prior processes mentioned, using a filter column having a reversible chemical effect, but the present invention employs a single developer comprising a solution of a substance which has a stronger affinity for the column material and is more strongly bound thereby than any of the components to be separated, whereby the components are displaced in turn according to their relative affinities for the column material. The invention will be further described by reference to synthentic resinous ion-exchange materials, which are used as a column packing in the form of particles graded by sieving.

The invention enables organic solutes of biological origin such as amino-acids, amino-sugars, and their derivatives to be cleanly separated, a matter of considerable difficulty by known methods. The solvent will in general be water but need not be and in some cases, as will be explained below, advantages are obtainable by the use of mixed solvents.

The solution containing the mixed solutes to be fractionated is passed at a uniform speed through the column, and those components or ions, acidic or basic, that are reactive towards the ion-exchanger used for packing the column, are absorbed or combined, while components or ions of opposite character or charge to those absorbed or electrically neutral substances flow out with the solvent. The solution is allowed to pass until a certain proportion of the ion-exchange material is saturated and will absorb no more, when the flow of solution is discontinued. At this point a boundary has formed some distance along the tube, which divides the saturated from the unsaturated portion of the column, and to some extent the absorbed or combined components of the solution have arranged themselves in order of decreasing affinity for the ion-exchanger, the most strongly combined components appearing in the first part of the column. A developer in the form of a solution containing an appropriate concentration of a substance which will be more strongly combined by the column material than any of the substances to be separated, and which may be either an organic or an inorganic acid or base, is then passed through the column at a regulated rate. This substance forms a sharply bounded band at the beginning of the column displacing the most strongly combined component of the original solution. This in turn displaces the next component, until a time is reached at which the system acquires a dynamic equilibrium and the components form a series of sharply separated bands, all moving along the column together. When the least strongly combined component reaches the end of the column it flows out with the solvent.

Since in general the components will not be coloured substances and the separate bands formed by the individual solutes of the column will not be visible, a means is necessary whereby the composition of the effluent liquid may be continuously observed, in order to determine at what time it is necessary to change the receiver collecting the effluent so as to separate clean fractions. For this purpose measurement of one or more of the physical properties of the components may be used. The most useful of these properties are electrical conductivity, hydrogen ion concentration and refractive index, but in particular cases other properties may be utilised; the most suitable physical properties to be measured must be chosen in relation to the properties of the components in the solution, each case being considered on its merits.

For the properties referred to, the apparatus is completed by the provision of suitable instruments of known kind for the measurement of electrical conductivity, hydrogen ion concentration and/or refractive index of the liquid flowing from the column. These instruments are arranged either to give a continuous indication to enable the operator to change the receiver as a new fraction appears or by means of switch-gear and mechanism of known kind to change the receiver automatically as a new fraction appears. The instruments may also be arranged to make a permanent record on a graph or photographic film of the changes in the properties measured. An arrangement may also be provided whereby the volume of each fraction may be measured or its weight observed.

As an alternative arrangement for following changes in composition of the effluent solution, the solution as it flows from the column may be divided into a large number of fractions of equal volume. Samples may then be taken from these fractions for the purpose of measuring physical properties such as electrical conductivity, p_H or refractive index, or for chemical analysis. One suitable analytical procedure is to titrate a measured sample from the fraction with standard alkali, if the fraction contains an acid or with standard acid if the fraction contains a base. Alternatively a qualitative analysis may be carried out on each fraction by means of the method of filter paper partition chromatography which is now well known. (Consden R., Gordon A.H. and Martin A. J. P. 1944 Biochen. J. 38, 224). This latter method is particularly well suited where the mixture to be separted is a protein hydrolysate, and thus contains a large number of amino-acids all of which may be identified by means of the filter paper partition chromatogram. The division of the effluent solution into fractions of equal volume may, for preference, be carried out automatically by means of one of the known machines designed for this purpose.

The ion-exchange material may be a substance which is capable of forming insoluble salt-like complexes with acidic components or ions, or may be similarly reactive with basic components or ions, and the insoluble salts so formed should possess the property of reversible dissociation, so that weakly reacting components or ions, acidic or basic, may be quantitatively displaced under the proper conditions by those which have a stronger affinity for the ion-exchange material. Synthetic resins prepared by the polymerization of smaller molecules having an acidic or basic function are suitable, and such resins are commercially obtainable products. For example the resin sold under the trade name " Zeo Karb 215 " (" Zeo-Karb " being a registered trade mark) has properties similar to those of a sulphonated phenol-formaldehyde resin, while that sold under the name " Deacidite B " has properties similar to those of a phenylene-diamine-formaldehyde resin.

The solution containing the mixture of substances to be separated, e.g. organic substances of biological origin such as amino-acids or their derivatives, may be an aqueous extract or may contain varying proportions of organic solvents miscible with water such as alcohol or acetone; the use of organic solvents in this way may be necessary where some of the components to be separated have low solubilities in water alone. A proportion of organic solvents may be mixed with the solution to be separated for the purpose of modifying the ionic dissociation of one or all of the components of the mixture in order to secure a better separation. For instance, the separation of serine and glutanic acid on a column of the cation exchanger " Zeo-Karb 215 " is not satisfactory where a purely aqueous solvent is used but the presence of acetone selectively modifies the dissociation of glutanic acid and the components may readily be separated on the same column when equal parts of acetone and water are used as the solvent.

Solvents which are not miscible with water may also be used with advantage for the purpose of particular separations. In this case since ion exchangers are hygroscopic, they contain water bound within the particles, and two liquid phases are set up within the column; a stationary water phase bound by the resin and the moving solvent phase which may also contain water dissolved in it. Under these circumstances if one of the components of the mixture to be separated has a more favourable partition coefficient between water and the solvent than the other components it will be preferentially dissolved in the solvent phase and will thus pass further down the column.

When the solution contains mixed organic and inorganic solutes, as is very common with extracts of biological origin, the solution may be first passed through a column containing a synthetic resin with acid properties which removes cations and leaves a solution containing free acids, both organic and inorganic, together with non-electrolytes. The effluent solution may then be passed through a column containing a synthetic resin with basic properties which removes the acids, the non-electrolytes flowing through with the solvent. Solutions more suitable for separation may then be obtained from the columns, by passage of a basic developer in the one case and an acid developer in the other: by suitable fractionation a solution containing organic acids only may be recovered from the column with basic properties and a similar fraction containing organic bases only may be obtained from the column with acid properties, the two solutions then being dealt with separately on the appropriate columns.

Where the original solution is acid in character it may be advantageous to reverse the above procedure and to pass the solution first through an anion-

exchanger to bind free acids and anions, and afterwards to pass it through a cation-exchanger to remove the residual cations. The two columns are then developed with the appropriate acidic or basic reagents, and if necessary fractions taken during development may be further fractionated on smaller columns.

It should be mentioned that efficiency of separation is directly related to the height of the band occupied by the individual to be separated. For good separation on laboratory columns this height should be as much as 10 cms. so that for solutions containing a large number of components the columns become inconveniently long. We find however that the desired effect can be obtained by attaching a narrow subsidiary column packed with fine material to the bottom of the main column. The main column can then be shorter and wider and is consequently more easily packed and handled.

The yield of a component obtained in pure condition from a single passage through the column is related to the sharpness of the boundaries of the bands formed by the components, and in general it is found that the boundaries are sharper, the lower the rate of flow of the solution through the column. Fine grinding of the ion-exchanger increases the sharpness of the boundaries; for example, where the base exchanger "Zeo-Karb 215" is used it has been found that for best results the resin should be ground and graded between 40 meshes per inch and 60 meshes per inch wire sieves.

Many commercial cation exchange resins contain, in their structure, phenolic hydroxyl groups in addition to the sulphonic acid radicles to which their properties as cation exchangers may be mainly attributed. The presence of phenolic hydroxyl groups is a disadvantage where the resin is applied to the separation of bases stronger than ammonia since under highly alkaline conditions, these groups react slowly with strong bases leading to diffuse boundaries. In the separation of strong bases it is therefore of advantage to use a cation exchange resin which does not contain an appreciable proportion of free phenolic hydroxyl radicles.

The following examples will further illustrate the nature of the invention:—

(1) A column of 9 mm. diameter and 10.5 cm. height was packed with 3 gms. of a synthetic cation exchange resin ("Zeo-Karb 215"—ground to 40—60 mesh) and mounted above an assembly consisting of a conductivity cell and a glass electrode cell, both arranged for the continuous measurement of the conductivity and *p*H of the effluent from the column, the two cells and a socket for the column all being built of poly-methylmethacrylate plastic parts, sealed together with petroleum jelly. The ion-exchange material was activated by the passage of 2N hydrochloric acid, and was then washed with distilled water until the conductivity and *p*H readings showed that the effluent was substantially free from hydrochloric acid.

A mixture containing creatinine (192 mgs.) and glycine (113 mgs.) dissolved in 20 ml. of water was then passed into the column. The substances were adsorbed in the upper part of the column and as both test substances had the property of modifying the brown colour of the ion-exchange material, their presence was shown by the formation of two bands, one of them orange-yellow in colour and the other red-brown. In order to remove traces of anions present as impurities in the test substances, the columns were then washed with distilled water (50 mls.) until the conductivity of the effluent fell to a value approaching that of the distilled water used for washing.

Ammonia solution (0.0213 N) was then passed into the column at a rate of 2.5 ml. per minute in order to displace the bands due to the test substances and to cause their passage down the column. The part of the column occupied by the ammonia was dark brown in colour, and as they passed down the column, the fronts due to the three substances were observed to become more sharp.

From the time the ammonia solution was applied to the first appearance of glycine in the effluent 275 mls. of substantially pure water flowed from the column. From then on to the final appearance of pure ammonia solution in the effluent, recordings of resistance in ohms across the conductivity cell and millivolts across the glass electrode cell were taken at intervals of 1 ml. and the results were plotted. The results are shown in Figure 1 of the accompanying drawings. It will be seen that as the glycine increased in concentration when its front reached the lower orifice of the volumn the resistance rose to 4.75×10^4 ohms. At this value it remained substantially stationary until the front due to a creatinine reached the orifice. A marked fall in resistance followed and resistance measurements again become substantially stationary at 2.27×10^4 ohms when the last of the glycine left the column and the effluent consisted of pure creatinine solution only. Similarly, the resistance again fell sharply and then reached a further stable level at 0.25×10^4 ohms as the

ammonia boundary reached the bottom of the column.

Since the test solutions were displaced in order of increasing strength as bases, the pH measurements increased in steps, as also shown in Figure 1. The shape of the pH curve is accordingly the inverse of that given by the measurements of resistance.

In order to verify the purity of the fractions lying under the flat portions of the curves, analyses were carried out on appropriate fractions of the effluent. The recovery of creatinine in a pure condition was 63.5 per cent. and the total recovery was 99 per cent.; while the recovery of pure glycine was 74.5 per cent., the total recovery being 100 per cent.

It should be pointed out that the percentage recovery of the pure components shown in this experiment should not be taken as an indication of the probable efficiency of larger scale applications of the method since the apparatus used was designed for the separation of small quantities of material as a laboratory micro-method. Efficiency is considerably improved by use of longer columns and larger quantities of material.

Figure 2 gives the results of an experiment in which a mixture of aspartic acid, glycine and histidine were separated by displacement with ammonia solution. The column used was 15 mm. in diameter and 400 mm. high. It contained 28 gm. (dry wt.) of " Zeo-Karb 215 " which had previously been ground to 40—60 meshes per inch and prepared according to the procedure indicated in Example 1. An aqueous solution (1 litre) containing aspartic acid (2.0 gms.), glycine (2.0 gms.) and histidine mono-hydrochloride (2.7 gms.) was passed through the column at a rate of 250 ml./hr. after which the column was washed with a further 500 ml. distilled water in order to ensure the removal of residual hydrochloric acid from the amino-acid hydrochloride.

Displacement was carried out with 0.10N ammonia solution (1210 ml.) which was introduced at a rate of 200 ml./hr., and the effluent was collected in 10 ml. fractions. The conductivity of the effluent was recorded continuously during the run, and the results are plotted in the form of a smooth curve in Figure 2. The figure also shows pH readings determined on every 4th fraction after the run was complete.

It will be seen from the form of the curves that the course of the fractionation can be followed from the pH and conductivity measurements alone, but in order to provide a more sensitive check on the purity of the fractions, a drop of the solution was taken from each 10 ml. fraction and introduced on to a filter paper chromatogram. This was irrigated with phenol and developed with ninhydrin according to the technique of Consden et al (1944). The resulting chromatogram is represented in Figure 2 in the form of a histogram. The areas enclosed by dotted lines at the leading and trailing edges of the amino-acid bands represent the presence of the amino-acid as a trace only. It is clear from the histogram, that the effluent collected in fractions 1—42 contained aspartic acid only, while that in fractions 56—92 and fractions 101—114 contained pure glycine and pure histidine respectively. Fractions 115—121 contained histidine and ammonia, but since the latter may readily be removed by evaporation under reduced pressure, the material is suitable for addition to the main histidine fraction.

Fractions 1—44, 56—92 and 101—121 were combined and in each case the solution was evaporated to small bulk under reduced pressure. The concentrated solutions were then evaporated to dryness under vacuum. A colourless crystalline product was obtained in each case, in the yields given below; the purity of the fraction was confirmed by analysis for nitrogen.

	Yield	Yield %	N%
Aspartic acid -	1.63 g.	81.5	10.5 ($C_4H_7O_4N$ requires 10.53)
Glycine - - -	1.54	77	18.7 ($C_2H_5O_2N$,, 18.67)
Histidine - -	1.77	80.5	27.0 ($C_6H_9O_2N_3$,, 27.10)

(3) A column was packed with 150 gms. of a sample of " Zeo-Karb 215 " that had been cycled several times between 2N.NaOH and 2N.HCl (diameter of column 2.0 cms., height of column 106 cms., particle size 20—40 mesh). Commercial egg albumin (10 g.) was hydrolised with 5.5N HCl (30 hrs.) and most of the volatile acid removed by evaporation under vacuum. The hydrolysate was introduced on to the column and NaOH solution (0.1 N) was then applied as the displacement developer at a rate of 7.5 ml. per minute. 4.7 litres of effluent passed before conductivity readings indicated break-through of amino-acids. The effluent was then collected in 160 fractions each of 10 mls. until the conductivity indicated the break-through of NaOH. Alternate fractions were tested by introducing spots of the liquid on to filter paper chromatograms, and on the basis of the qualitative analysis so obtained the effluent fractions were

grouped to form 6 main fractions containing (1) aspartic acid and traces of serine. (2) glutamic acid, serine and threonine plus a small quantity of aspartic acid. (3) glycine and alanine plus small quantities of glutamic acid and valine. (4) alanine, valine, the leucines, proline, cystine, methionine, phenylalanine and tyrosine plus traces of histidine. (5) a fraction rich in histidine. (6) a fraction rich in lysine.

These fractions were further differentiated (a) by a second passage through a cation exchange column using the same resin or a modified resin, (b) the use of aqueous-organic solvents which have the effect of differentially modifying the ionisation of the ampholytes, (c) the use of anion-exchangers. Thus pure samples of aspartic acid, histidine and lysine were obtained from fractions (1), (5) and (6) respectively by application of method (a). Fraction (2) on further fractionation of a "Zeo-Karb" column using 50% acetone-NH_3 as displacement developer yielded aspartic acid, glutamic acid and serine in a pure state. A second portion of fraction (2) was passed down a column of the commercial anion-exchange resin "Deacidite B". A mixed fraction containing serine and threonine passed through the column without being adsorbed by the resin, while glutamic acid was retained. Pure glutamic acid was recovered by displacement with 0.1N hydrochloric acid solution.

In cases where it is desired to isolate particular amino acids from the hydrolysates of natural proteins, it is advantageous to choose a protein in which the amino acid is especially abundant. Thus, the protein mixture "gluten" obtained from wheat is a particularly good source of glutamic acid and high yields of this amino acid are obtainable by the method given in example, (3) from the acid hydrolysis product of the wheat protein.

Similarly a protein may be chosen because of the absence in it of a component which interferes with the separation of a desired amino acid. For instance, the proteins salmine and clupeine, prepared from the sperm of the salmon and the herring respectively, do not contain all the members of the mono amino monocarboxylic acid group, which are difficult to separate one from another. These two proteins are composed largely of arginine, serine, valine and proline and on hydrolysis yield a mixture which may be separated into the constituent amino acids by the techniques illustrated in example (3).

Having now particularly described and ascertained the nature of our said invention and in what manner the same is to be performed, we declare that what we claim is:—

1. A method of fractionation of solutions containing mixed solutes consisting at least in part of organic solutes of biological origin which includes the steps of passing the solution into a column of a particulate ion-exchange material wherein components to be separated are chemically bound and then displacing said bound components out of the column in turn by passing into the column a single developer comprising a solution of a substance which is more strongly bound by the column material than any of the components to be displaced, whereby the components are displaced in turn according to their relative affinities for the column material.

2. A method of fractionation of solutions containing mixed solutes consisting at least in part of organic solutes of biological origin which includes the steps of passing the solution into a first column of a particulate ion-exchange material wherein some of the components to be separated are chemically bound, passing the effluent from the first column into a second column of a particulate ion-exchange material of opposite character to that in the first column wherein other of the components to be separated are chemically bound, and displacing the fractions in turn out of the two columns by passing into each a single respective solution of a substance which is more strongly bound by the respective column material than is any of the said components chemically bound therein, whereby the components are displaced in turn according to their relative affinities for the respective column materials.

3. A method according to claim 2 in which the first column consists of a material with acid properties which binds cations and the second column consists of a material with basic properties which removes acids from the solution.

4. A method according to claim 1 or 3 in which the column material is a synthetic resin.

5. A method according to any preceding claim wherein the solvent in the solution treated is a mixture of water and an organic solvent which modifies the ionic dissociation of at least one of the solutes to be separated in such a way as to differentiate its affinity for the column material more strongly in relation to other of the solutes.

6. A method according to any of claims 1 to 4 wherein the solvent in the solution treated is a mixture of water and an organic solvent such that at least one of

the solutes to be separated has a more favorable partition coefficient between water and the said solvent than have other of the solutes.

7. A method according to any preceding claim in which the mixed solutes include strong bases, in which the column consists of a cation-exchange synthetic resin substantially free from phenolic hyroxyl radicles and in which the displacing solution is of a base stronger than the strongest basic solute bound in the column.

8. A method according to any preceding claim in which the displaced solutes are sorted by determination of a physical property which differs for the several displaced solutes.

9. A method as claimed in claim 8 in which the physical property by the aid of which sorting is effected is electrical conductivity.

10. A method according to any preceding claim as applied to an aqueous solution containing hydrolised proteins, the column consisting of ion-exchange synthetic resin of acid character.

11. A method according to claim 10 wherein the displacing base is ammonia.

12. A method according to claim 10 as applied to an aqueous solution of mixed amino acids, the column consisting of ion-exchange synthetic resin of alkaline character.

13. A method according to claim 12 wherein the displacing solution is hydrochloric acid.

Dated this 31st day of May, 1948.

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[This Drawing is a reproduction of the Original on a reduced scale.]

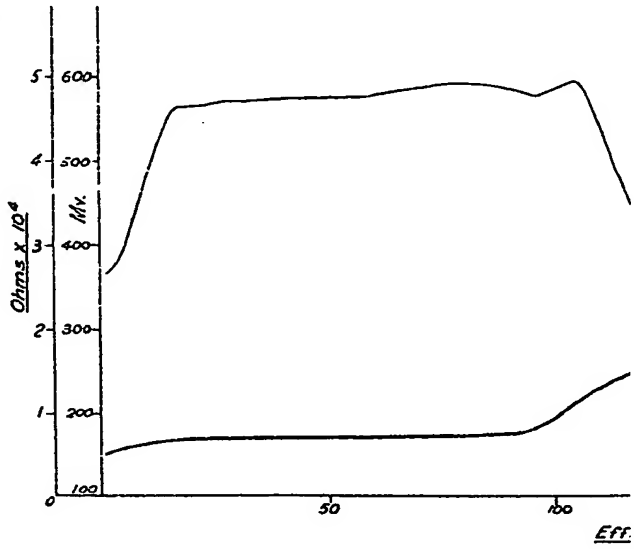
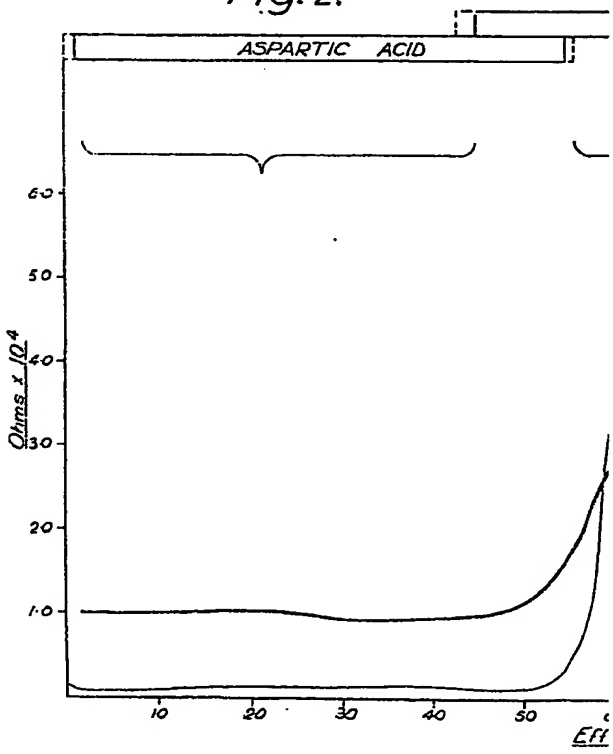


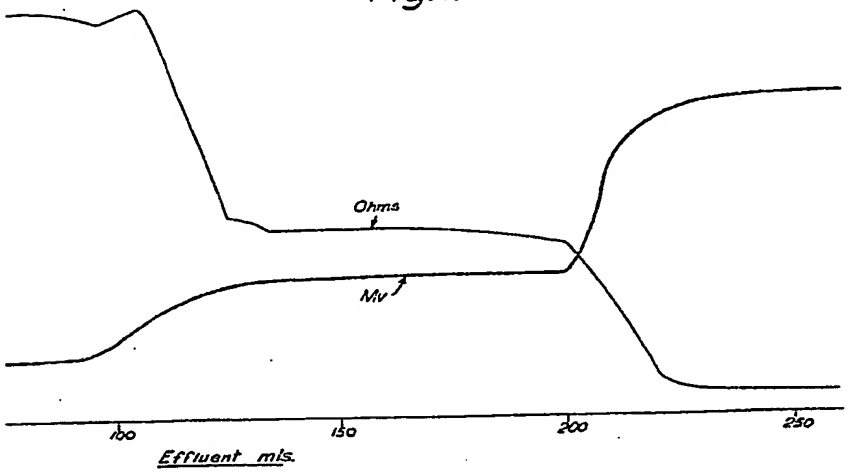
Fig. 2.



2 SHEETS

SHEET 1

Fig. 1.



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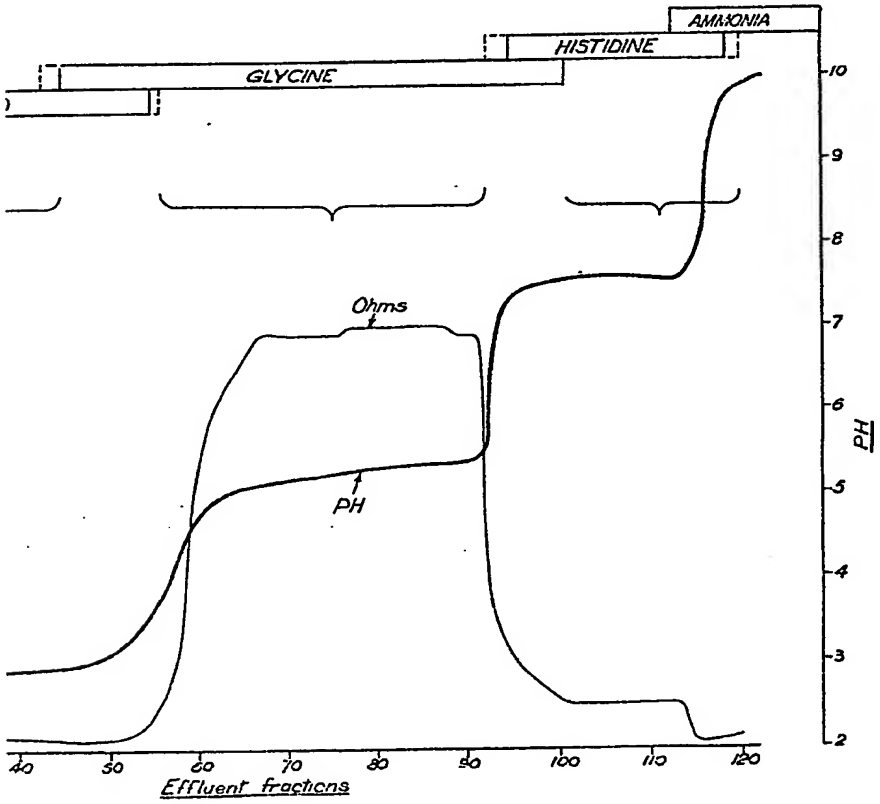


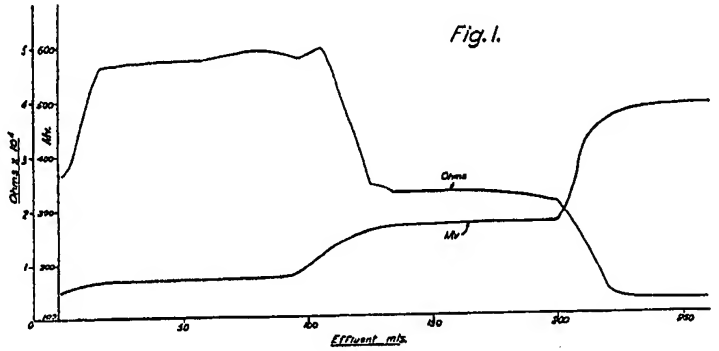
H.M.S.O. (Ty. P.)

644,382 COMPLETE SPECIFICATION

2 SHEETS

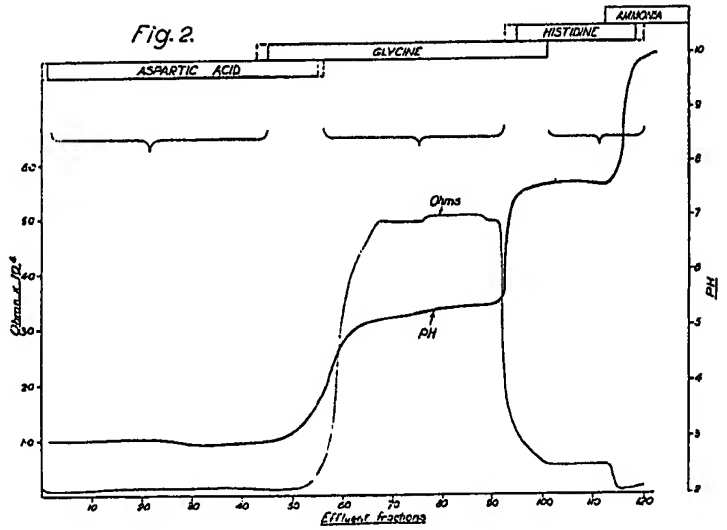
SHEET 1

Fig. 1.



SHEET 2

Fig. 2.



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H.M.S.O. (Ty. 4)